

DESIGN OF SYNTHETIC DNA PROBES.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15														
A.	M	-	N	-	P	-	N	-	N	-	R	-	S	-	E	-	H	-	D	-	T	-	I	-	K	-	T	-	T
	ATG	AAT	CCN	AAT	AAT	CGN	TCN	GAA	CAT	GAT	ACN	ATT	AAA	ACN	ACN														
			C		C	C	AGA	AGT	G	C	C		C	G															
							G	C						A															

B. ATGAATCCTAATAATCG
 C C C C
 A
 G

C. GAACATGATACAATTAA
 G C C G C
 A

- A. PROTEIN SEQUENCE OF THE N-TERMINI OF PEAKS A AND B OF THE B.T.T. TOXIN AND DEDUCED DNA SEQUENCE.
- B. SYNTHETIC A1 PROBE, 32-FOLD DEGENERATE 17-MER, BASED ON AMINO ACIDS 1-6.
- C. SYNTHETIC A2 PROBE, 48-FOLD DEGENERATE 17-MER, BASED ON AMINO ACIDS 8-13.

FIG.1

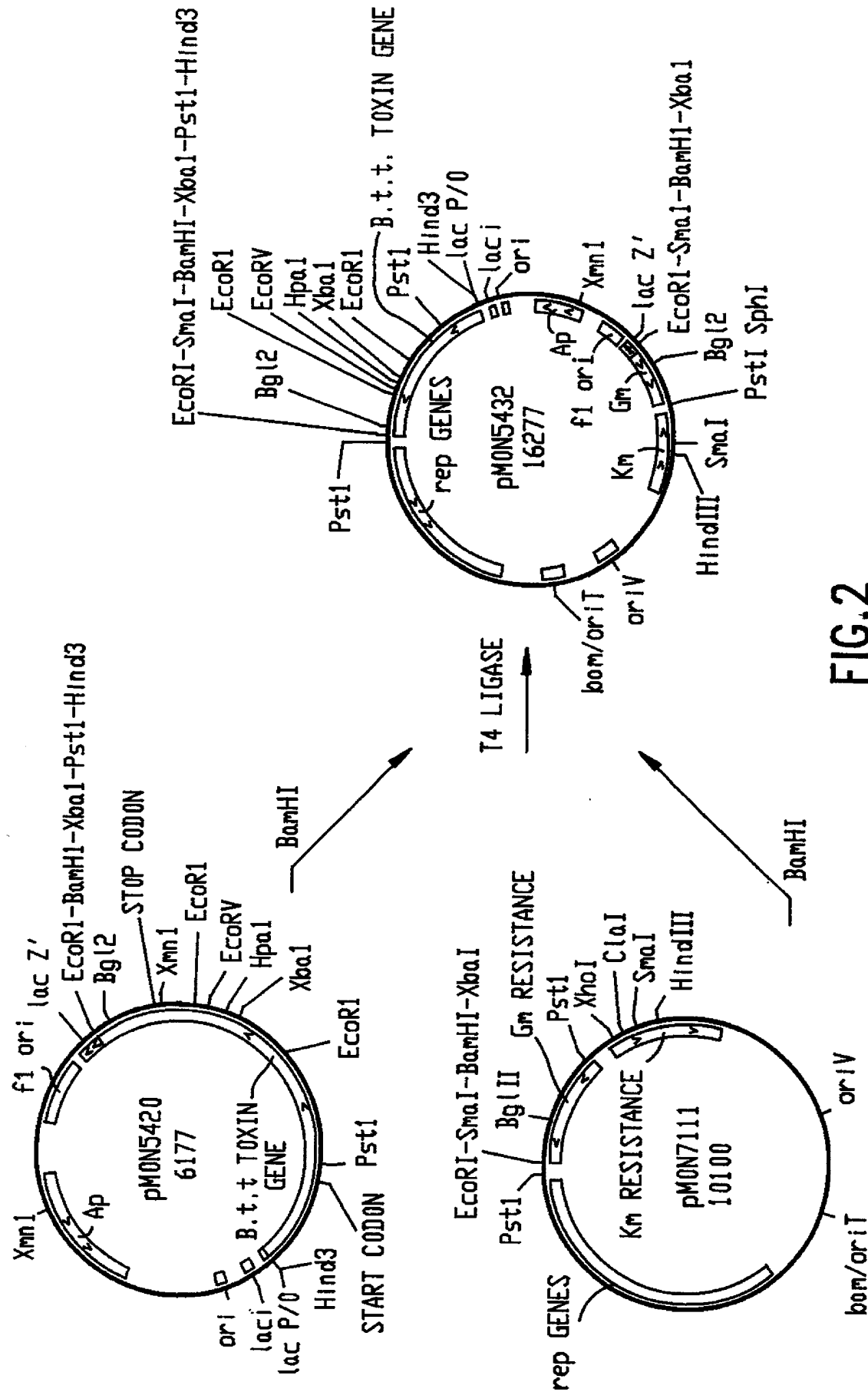


FIG.2

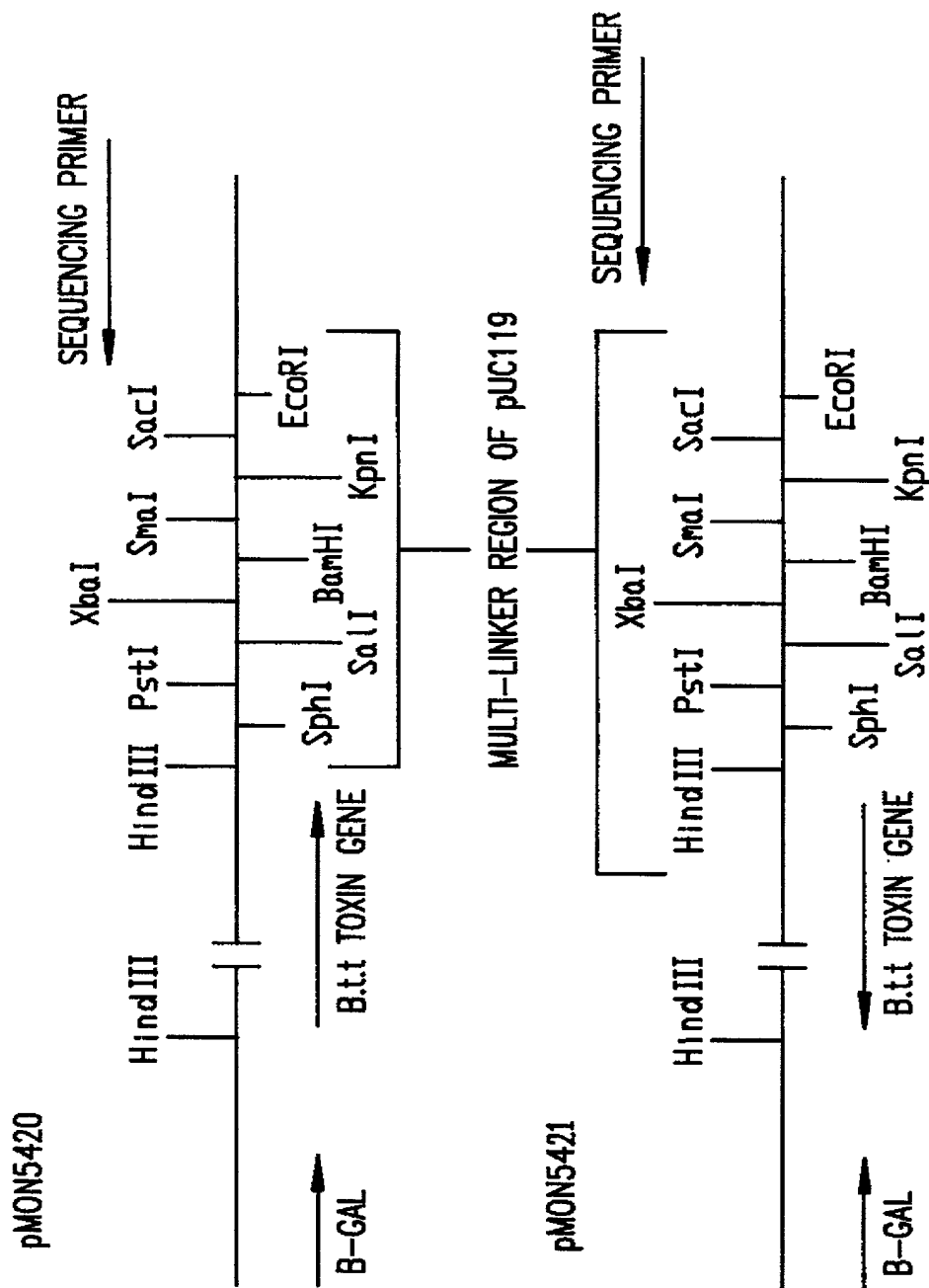


FIG.3

SEQUENCING OF THE *Bacillus Thuringiensis* var. *Tenebrionis*

INSECTICIDAL TOXIN GENE

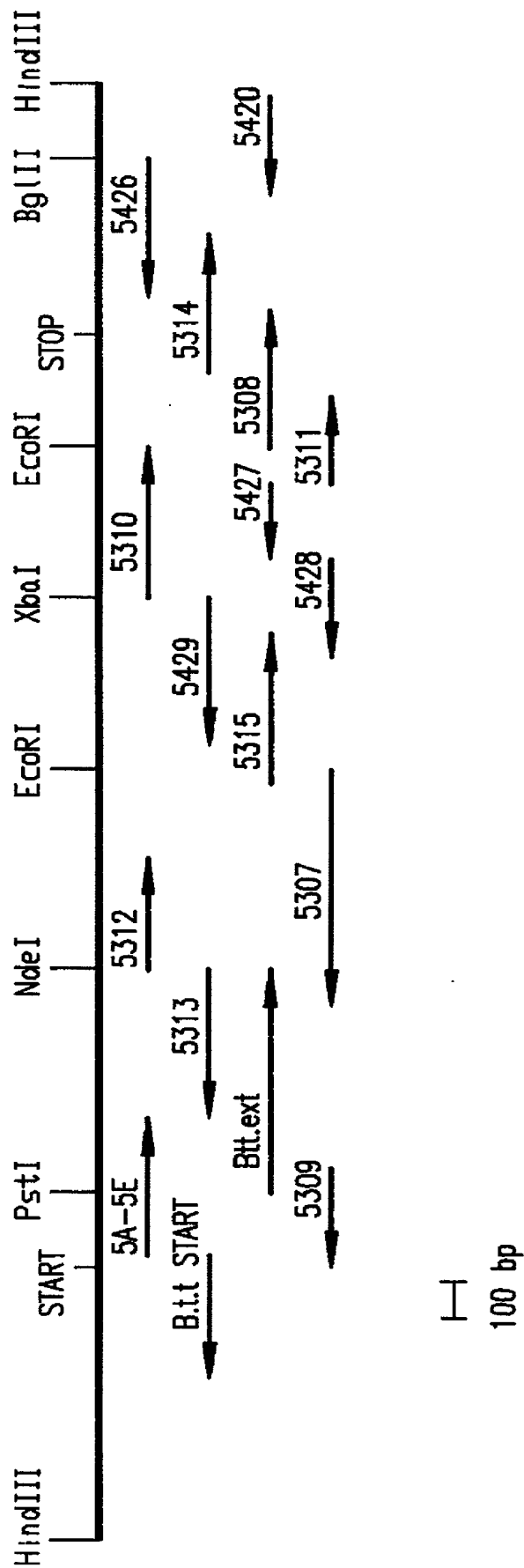


FIG.4

SEQUENCE OF THE B.t.t. INSECTICIDAL TOXIN GENE AND FLANKING REGIONS

H
i
n
f
1

gagcgactattataatcatatcatatctTATTGGAATGATTAAGATTCCAATAGAATAG
1 -----+-----+-----+-----+-----+-----+ 60
ctcgtgataatattagtatgtataaaagATAACCTTACTAATTCTAAGGTTATCTTATC

S
M f F M
n a o b
l N k o
1 1 1 2

TGTATAAATTATTTATCTTGAAAGGAGGGATGCCTAAAAACGAAGAACATTAAAAACATA
61 -----+-----+-----+-----+-----+-----+ 120
ACATATTTAATAAATAGAACTTTCCTCCCTACGGATTTTGTCTTCTTGTAATTTTGTAT

TATTTGCACCGTCTAATGGATTTATGAAAAATCATTTTATCAGTTTGAAAATTATGTATT
121 -----+-----+-----+-----+-----+-----+ 180
ATAAACGTGGCAGATTACCTAAATACTTTTGTAGTAAATAGTCAAACCTTTTAATACATAA

H
M i M T N
n n b a l
l f o q a
1 1 2 1 3

ATGATAAGAAAGGGAGGAAGAAAAATGAATCCGAACAATCGAAGTGAACATGATACAATA
181 -----+-----+-----+-----+-----+-----+ 240
TACTATTCTTTCCCTCCTTCTTTTACTTAGGCTTGTTAGCTTCACTTGTTACTATGTTAT

START M N P N N R S E H D T I -

FIG.5A

12345678910111213141516171819202122232425262728293031323334353637383940414243444546474849505152535455565758596061626364656667686970717273747576777879808182838485868788899091929394959697989910010110210310410510610710810911011111211311411511611711811912012112212312412512612712812913013113213313413513613713813914014114214314414514614714814915015115215315415515615715815916016116216316416516616716816917017117217317417517617717817918018118218318418518618718818919019119219319419519619719819920020120220320420520620720820921021121221321421521621721821922022122222322422522622722822923023123223323423523623723823924024124224324424524624724824925025125225325425525625725825926026126226326426526626726826927027127227327427527627727827928028128228328428528628728828929029129229329429529629729829930030130230330430530630730830931031131231331431531631731831932032132232332432532632732832933033133233333433533633733833934034134234334434534634734834935035135235335435535635735835936036136236336436536636736836937037137237337437537637737837938038138238338438538638738838939039139239339439539639739839940040140240340440540640740840941041141241341441541641741841942042142242342442542642742842943043143243343443543643743843944044144244344444544644744844945045145245345445545645745845946046146246346446546646746846947047147247347447547647747847948048148248348448548648748848949049149249349449549649749849950050150250350450550650750850951051151251351451551651751851952052152252352452552652752852953053153253353453553653753853954054154254354454554654754854955055155255355455555655755855956056156256356456556656756856957057157257357457557657757857958058158258358458558658758858959059159259359459559659759859960060160260360460560660760860961061161261361461561661761861962062162262362462562662762862963063163263363463563663763863964064164264364464564664764864965065165265365465565665765865966066166266366466566666766866967067167267367467567667767867968068168268368468568668768868969069169269369469569669769869970070170270370470570670770870971071171271371471571671771871972072172272372472572672772872973073173273373473573673773873974074174274374474574674774874975075175275375475575675775875976076176276376476576676776876977077177277377477577677777877978078178278378478578678778878979079179279379479579679779879980080180280380480580680780880981081181281381481581681781881982082182282382482582682782882983083183283383483583683783883984084184284384484584684784884985085185285385485585685785885986086186286386486586686786886987087187287387487587687787887988088188288388488588688788888989089189289389489589689789889990090190290390490590690790890991091191291391491591691791891992092192292392492592692792892993093193293393493593693793893994094194294394494594694794894995095195295395495595695795895996096196296396496596696796896997097197297397497597697797897998098198298398498598698798898999099199299399499599699799899910001001100210031004100510061007100810091010101110121013101410151016101710181019102010211022102310241025102610271028102910301031103210331034103510361037103810391040104110421043104410451046104710481049105010511052105310541055105610571058105910601061106210631064106510661067106810691070107110721073107410751076107710781079108010811082108310841085108610871088108910901091109210931094109510961097109810991100110111021103110411051106110711081109111011111112111311141115111611171118111911201121112211231124112511261127112811291130113111321133113411351136113711381139114011411142114311441145114611471148114911501151115211531154115511561157115811591160116111621163116411651166116711681169117011711172117311741175117611771178117911801181118211831184118511861187118811891190119111921193119411951196119711981199120012011202120312041205120612071208120912101211121212131214121512161217121812191220122112221223122412251226122712281229123012311232123312341235123612371238123912401241124212431244124512461247124812491250125112521253125412551256125712581259126012611262126312641265126612671268126912701271127212731274127512761277127812791280128112821283128412851286128712881289129012911292129312941295129612971298129913001

D	BH	M
r	aa	b
a	le	o
1	13	2
	/	

E	S			
c	a	A	A	M
o	u	l	l	a
p	3	u	u	e
1	B A	1	1	3

FIG. 5C

S			
DqH	T	M	N
rua	a	b	l
a9e	q	o	a
263	1	2	3
/			

TTACAGGGCCTTCAAAATAATGTCGAAGATTATGTGAGTGCATTGAGTTCATGGCAAAA
 601 -----+-----+-----+-----+-----+ 660
 AATGTCCCGGAAGTTTTATTACAGCTTCTAATACTCACGTAAGTCAAGTACCGTTTT

 L Q G L Q N N V E D Y V S A L S S W Q K -

	BS	
	sc	A
	tr	l
	NF	u
	11	1
	/	

AATCCTGTGAGTTCACGAAATCCACATAGCCAGGGCGGATAAGAGAGCTGTTTTCTCAA
 661 -----+-----+-----+-----+-----+ 720
 TTAGGACACTCAAGTGCTTTAGGTGTATCGGTCCCCGCCTATTCTCTCGACAAAAGAGTT

 N P V S S R N P H S Q G R I R E L F S Q -

	M
	n
	l
	1

GCAGAAAGTCATTTTCGTAATTCATGCCTTCGTTTGCAATTTCTGGATACGAGGTTCTA
 721 -----+-----+-----+-----+-----+ 780
 CGTCTTTCAGTAAAAGCATTAAGTTACGGAAGCAAACGTTAAAGACCTATGCTCCAAGAT

 A E S H F R N S M P S F A I S G Y E V L -

FIG.5D

			F	
	B	N	An	
	b	d	Lu	
	v	e	u4	
	1	1	1H	

TTTCTAACACATATGCACAAGCTGCCAACACACATTTATTTTACTAAAAGACGCTCAA
 781 -----+-----+-----+-----+-----+-----+ 840
 AAAGATTGTTGTATACGTGTTTCGACGGTTGTGTGTAAATAAAAAATGATTTTCTGCGAGTT

 F L T T Y A Q A A N T H L F L L K D A Q -

H		M		M
g		b		b
a		o		o
1		2		2

 ATTTATGGAGAAGAATGGGGATACGAAAAAGAAGATATTGCTGAATTTTATAAAAGACAA
 841 -----+-----+-----+-----+-----+-----+ 900
 TAAATACCTCTTCTTACCCCTATGCTTTTCTTCTATAACGACTTAAATATTTTCTGTT

 I Y G E E W G Y E K E D I A E F Y K R Q -

		M		M
		a		n
		e		i
		2		1

 CTAAACTTACGCAAGAATATACTGACCATTGTGTCAAATGGTATAATGTTGGATTAGAT
 901 -----+-----+-----+-----+-----+-----+ 960
 GATTTTGAATGCGTTCTTATATGACTGGTAACACAGTTTACCATATTACAACCTAATCTA

 L K L T Q E Y T D H C V K W Y N V G L D -

		H	
		i	
		n	
		f	
		1	

 AAATTAAGAGGTTTCATCTTATGAATCTTGGGTAACTTTAACCGTTATCGCAGAGAGATG
 961 -----+-----+-----+-----+-----+-----+ 1020
 TTTAATTCTCCAAGTAGAATACTTAGAACCCATTTGAAATTGGCAATAGCGTCTCTCTAC

 K L R G S S Y E S W V N F N R Y R R E M -

FIG.5E

ACATTAACAGTATTAGATTTAATTGCACTATTTCCATTGTATGATGTTCCGGCTATACCCA
 1021 -----+-----+-----+-----+-----+-----+ 1080
 TGTAAATTGTCATAATCTAAATTAACTGTGATAAAGGTAACATACTACAAGCCGATATGGGT
 T L T V L D L I A L F P L Y D V R L Y P -

S H H
 aX i i
 uh n n
 3o f c
 A2 1 2
 /

AAAGAAGTTAAAACCGAATTAACAAGAGACGTTTAAACAGATCCAATTGTCGGAGTCAAC
 1081 -----+-----+-----+-----+-----+ 1140
 TTTCTTCAATTTTGGCTTAATTGTTCTCTGCAAAATTGTCTAGGTTAACAGCCTCAGTTG
 K E V K T E L T R D V L T D P I V G V N -

DM AT.
 ds sa
 et uq
 12 21
 /

AACCTTAGGGGCTATGGAACAACTTCTCTAATATAGAAAATTATTCGAAAACCAT
 1141 -----+-----+-----+-----+-----+ 1200
 TTGGAATCCCGATACCTTGTGGAAGAGATTATATCTTTTAATATAAGCTTTTGGTGTA
 N L R G Y G T T F S N I E N Y I R K P H -

E BS
 c T N sc
 o h l tr
 R a a NF
 1 1 4 11
 /

CTATTTGACTATCTGCATAGAATTCAATTTACACGCGGTTCCAACCAGGATATTATGGA
 1201 -----+-----+-----+-----+-----+ 1260
 GATAAACTGATAGACGTATCTTAAGTTAAAGTGTGCGCCAAGGTTGGTCTATAATACCT
 L F D Y L H R I Q F H T R F Q P G Y Y G -

FIG.5F

H	S	S
i	Aa H	M
n	vu p	a
f	a9 a	e
1	26 2	1
	/	

AATGACTCTTTCAATTATTGGTCCGGTAATTATGTTTCAACTAGACCAAGCATAGGATCA
 1261 -----+-----+-----+-----+-----+ 1320
 TTACTGAGAAAGTTAATAACCAGGCCATTAATACAAAGTTGATCTGGTTCGTATCCTAGT

N D S F N Y W S G N Y V S T R P S I G S -

E	P	
c	f	R
o	l	S
P	M	a
1	1	1

AATGATATAATCACATCTCCATTCTATGGAAATAAATCCAGTGAACCTGTACAAAATTTA
 1321 -----+-----+-----+-----+-----+ 1380
 TTACTATATTAGTGTAGAGGTAAGATACCTTTATTTAGGTCACTTGGACATGTTTTAAAT

N D I I T S P F Y G N K S S E P V Q N L -

E	
c	H
o	a
P	e
1	3

GAATTTAATGGAGAAAAAGTCTATAGAGCCGTAGCAAATACAAATCTTGCGGTCTGGCCG
 1381 -----+-----+-----+-----+-----+ 1440
 CTTAAATTACCTCTTTTTTCAGATATCTCGGCATCGTTTATGTTTAGAACGCCAGACCGGC

E F N G E K V Y R A V A N T N L A V W P -

FIG.5G

		M				S
		a				Ba
		e				cu
		3				13
						1A
						/

TCCGCTGTATATTCAGGTGTTACAAAAGTGAATTTAGCCAATATAATGATCAAACAGAT
 1441 -----+-----+-----+-----+-----+-----+ 1500
 AGGCGACATATAAGTCCACAATGTTTTACCTTAAATCGGTTATATTACTAGTTTGTCTA

S A V Y S G V T K V E F S Q Y N D Q T D -

			H			H
R		R	i		HT	AP
s		s	n		hh	lv
a		a	f		aa	uu
1		1	1		11	12
					/	/

GAAGCAAGTACACAAACGTACGACTCAAAAAGAAATGTTGGCGCGGTGAGCTGGGATTCT
 1501 -----+-----+-----+-----+-----+ 1560
 CTTGTTTCATGTGTTTGCATGCTGAGTTTTCTTTACAACCGCGCCAGTCGACCTAAGA

E A S T Q T Y D S K R N V G A V S W D S -

S				
CaT		M	XM	M
lua		n	ba	n
a3q		l	ae	l
1A1		1	11	1
/				

ATCGATCAATTGCCTCCAGAAACAACAGATGAACCTCTAGAAAAGGGATATAGCCATCAA
 1561 -----+-----+-----+-----+-----+ 1620
 TAGCTAGTTAACGGAGGTCTTTGTTGTCTACTTGGAGATCTTTTCCCTATATCGGTAGTT

I D Q L P P E T T D E P L E K G Y S H Q -

FIG.5H

	E		F	
	c		n	M
	o		u	a
	R		4	e
	V		H	3

ACAGGAGGAGATATCATTCAATGCACAGAAAATGGAAGTGGGCAACTATTTACGTTACA

1801 -----+-----+-----+-----+-----+ 1860

TGTCCTCCTCTATAGTAAGTTACGTGTCTTTACCTTCACGCCGTTGATAAATGCAATGT

T G G D I I Q C T E N G S A A T I Y V T -

				E	
H	R F	T	AM	c	D
p	s o	a	la	o	d
a	a k	q	ue	R	e
2	1 1	1	11	1	1

CCGGATGTGTCGTACTCTCAAAAATATCGAGCTAGAATTCATTATGCTTCTACATCTCAG

1861 -----+-----+-----+-----+-----+ 1920

GGCCTACACAGCATGAGAGTTTTATAGCTCGATCTTAAGTAATACGAAGATGTAGAGTC

P D V S Y S Q K Y R A R I H Y A S T S Q -

		B	
D	B Ns	T	
d	a lp	a	
e	n a1	q	
1	1 42	1	

ATAACATTTACACTCAGTTTAGACGGGGCACCATTTAATCAATACTATTTGATAAAACG

1921 -----+-----+-----+-----+-----+ 1980

TATTGTAAATGTGAGTCAAATCTGCCCGTGGTAAATTAGTTATGATAAAGCTATTTTGC

I T F T L S L D G A P F N Q Y Y F D K T -

ATAAATAAAGGAGACACATTAACGTATAATTCATTTAATTTAGCAAGTTTCAGCACACCA

1981 -----+-----+-----+-----+-----+ 2040

TATTTATTTCTCTGTGTAATTGCATATTAAGTAAATTAATCGTTCAAAGTCGTGTGGT

I N K G D T L T Y N S F N L A S F S T P -

FIG.5J

AT	H	AM
sa	g	ha
uq	a	ae
21	1	23
/		

2041 TTCTGAATTATCAGGGAATAACTTACAAATAGGCGTCACAGGATTAAGTGCTGGAGATAAA
 -----+-----+-----+-----+-----+ 2100
 AAGCTTAATAGTCCCTTATTGAATGTTTATCCGCAGTGTCCTAATTCACGACCTCTATTT

 F E L S G N N L Q I G V T G L S A G D K -

	X	M
	m	a
	n	e
	1	1

2101 GTTTATATAGACAAAATTGAATTTATTCCAGTGAATTAATTAAGTAAAGTAAAGAAG
 -----+-----+-----+-----+-----+ 2160
 CAAATATATCTGTTTTAACTTAAATAAGGTCACCTAATTTAATTGATCTTTCATTTCTTC

 V Y I D K I E F I P V N x STOP

M	M
a	b
e	o
3	2

2161 TAGTGACCATCTATGATAGTAAGCAAAGGATAAAAAAATGAGTTCATAAAATGAATAACA
 -----+-----+-----+-----+-----+ 2220
 ATCACTGGTAGATACTATCATTCGTTTCCTATTTTTTTACTCAAGTATTTTACTTATTGT

	M
	b
	o
	2

2221 TAGTGTTCTTCAACTTTTCGCTTTTTGAAGGTAGATGAAGAACACTATTTTTATTTTCAAA
 -----+-----+-----+-----+-----+ 2280
 ATCACAAGAAGTTGAAAGCGAAAACTTCCATCTACTTCTTGTGATAAAAAATAAAAGTTT

FIG.5K

	D		D	
	r		r	
	a		a	
	1		1	

2281 ATGAAGGAAGTTTTAAATATGTAATCATTTAAAGGGAACAATGAAAGTAGGAAATAAGTC
 -----+-----+-----+-----+-----+-----+-----+ 2340
 TACTTCCTTCAAAATTTATACATTAGTAAATTTCCCTTGTTACTTTCATCCTTTATTCAG

			S	
			s	
			p	
			1	

2341 ATTATCTATAACAAAATAACCATTTTTATATAGCCAGAAATGAATTATAATATTAATCTT
 -----+-----+-----+-----+-----+-----+-----+ 2400
 TAATAGATATTGTTTTATTGGTAAAAATATATCGGTCTTTACTTAATATTATAATTAGAA

				H	
		A	D	iH	S
		l	d	ng	s
		u	e	fa	p
		1	1	11	1

2401 TTCTAAATTGACG†TTTTCTaAACGTTCTATAGCTTCAAGACGCTTAGAATCATCAATAT
 -----+-----+-----+-----+-----+-----+-----+ 2460
 AAGATTTAACTGCaAAAAGA†TTGCAAGATATCGAAGTTCTGCGAATCTTAGTAGTTATA

				H	
A	A	T		i	B
c	l	a		n	g
c	u	q		f	l
1	1	1		1	2

2461 TTGTATACAGAGCTGTTGTTTCCATCGAGTTATGTCCCATTTGATTGCTAATAGAACAA
 -----+-----+-----+-----+-----+-----+-----+ 2520
 AACATATGTCTCGACAACAAAGGTAGCTCAATACAGGGTAAACTAAGCGATTATCTTGTT

FIG.5L

S			
aX		M	F
uh		n	o
3o		l	k
A2		1	1
//			

GATCTTTATTTTCGTTATAATGATTGGTTCATAAGTATGGCGTAATTTATGAGGGCTTT

2521 -----+-----+-----+-----+-----+-----+ 2580

CTAGAAATAAAAGCAATATTACTAACCAACGTATTCATACCGCATTAAATACTCCCGAAA

TCTTTTCATCCAAAAGCCAAGTGTATTTCTCTGTA

2581 -----+-----+-----+----- 2615

AGAAAAGTAGGTTTTTCGGTTCACATAAAGAGACAT

FIG.5M

B.t.t. PROTEINS – NATIVE & <u>E.coli</u> CLONES					
BAND No.	MW (Kd)	NATIVE B.t.t.	E.coli CLONES		
			pMON5436	pMON5456 pMON5450	pMON5460
1	73	—	—		—
2	71	—			
3,3'	67	—	—	—	
4	66	—			
DIAGRAM OF B.t.t. PROTEINS. B.t.t. PROTEINS PRODUCED BY <i>Bacillus Thuringiensis</i> var. <i>Tenebrionis</i> and <i>E.coli</i> JM101 (pMON5436, pMON5456, pMON5450, pMON5460) WERE RESOLVED ON 9% SDS-PAGE AND THE RESPECTIVE PATTERNS ARE SHOWN.					

FIG.6

FIG.7

N-TERMINI OF THE UNIQUE B.t.t. PROTEINS PRODUCED IN EITHER B.t.t. AND/OR E. COLI WERE DETERMINED BY AMINO ACID SEQUENCING. THE ARROWS AND ASSOCIATED NUMBERS CORRESPOND TO THE FIRST AMINO ACID OF THE PROTEINS DESCRIBED IN FIG. 6.

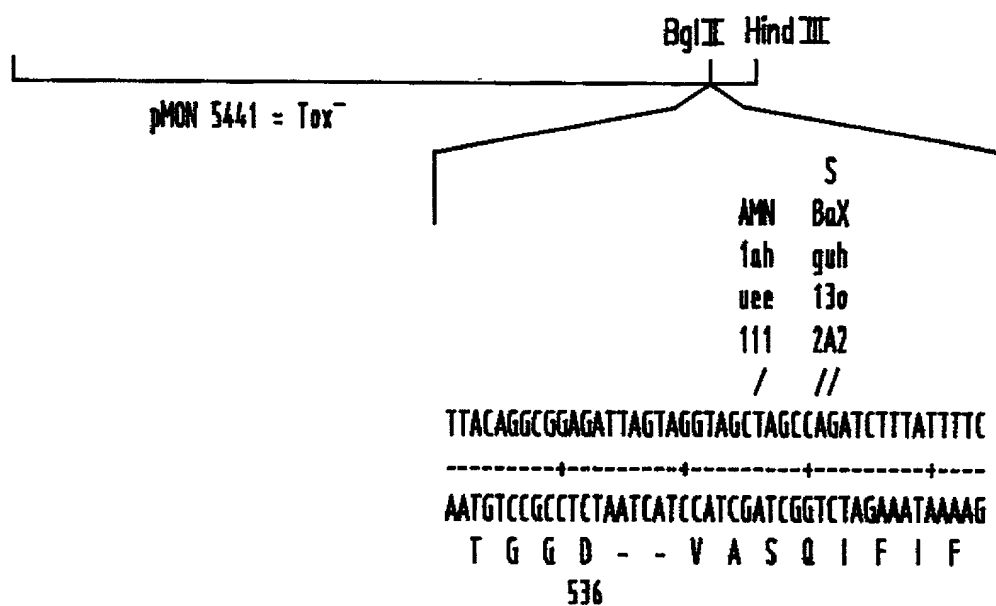
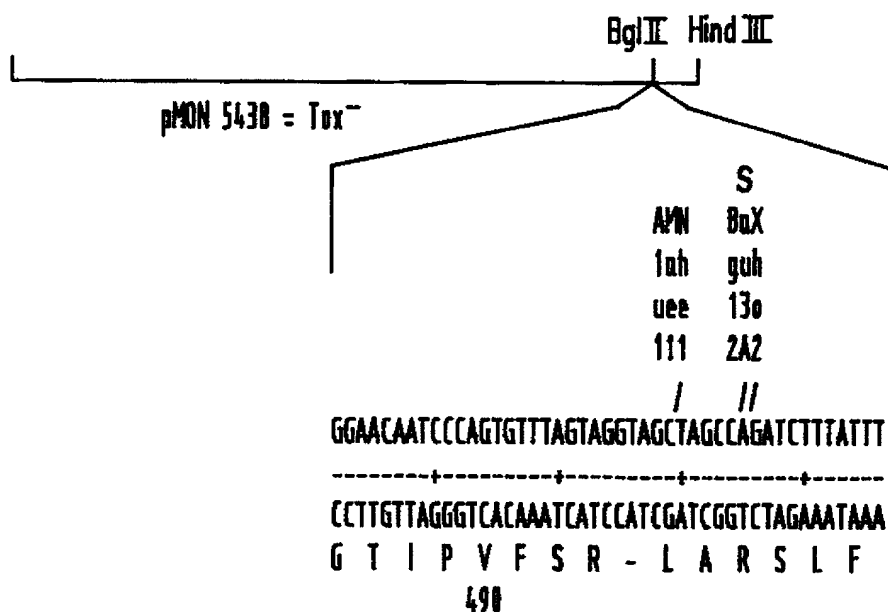
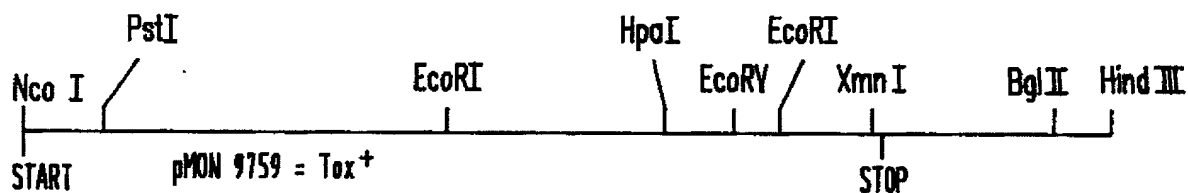
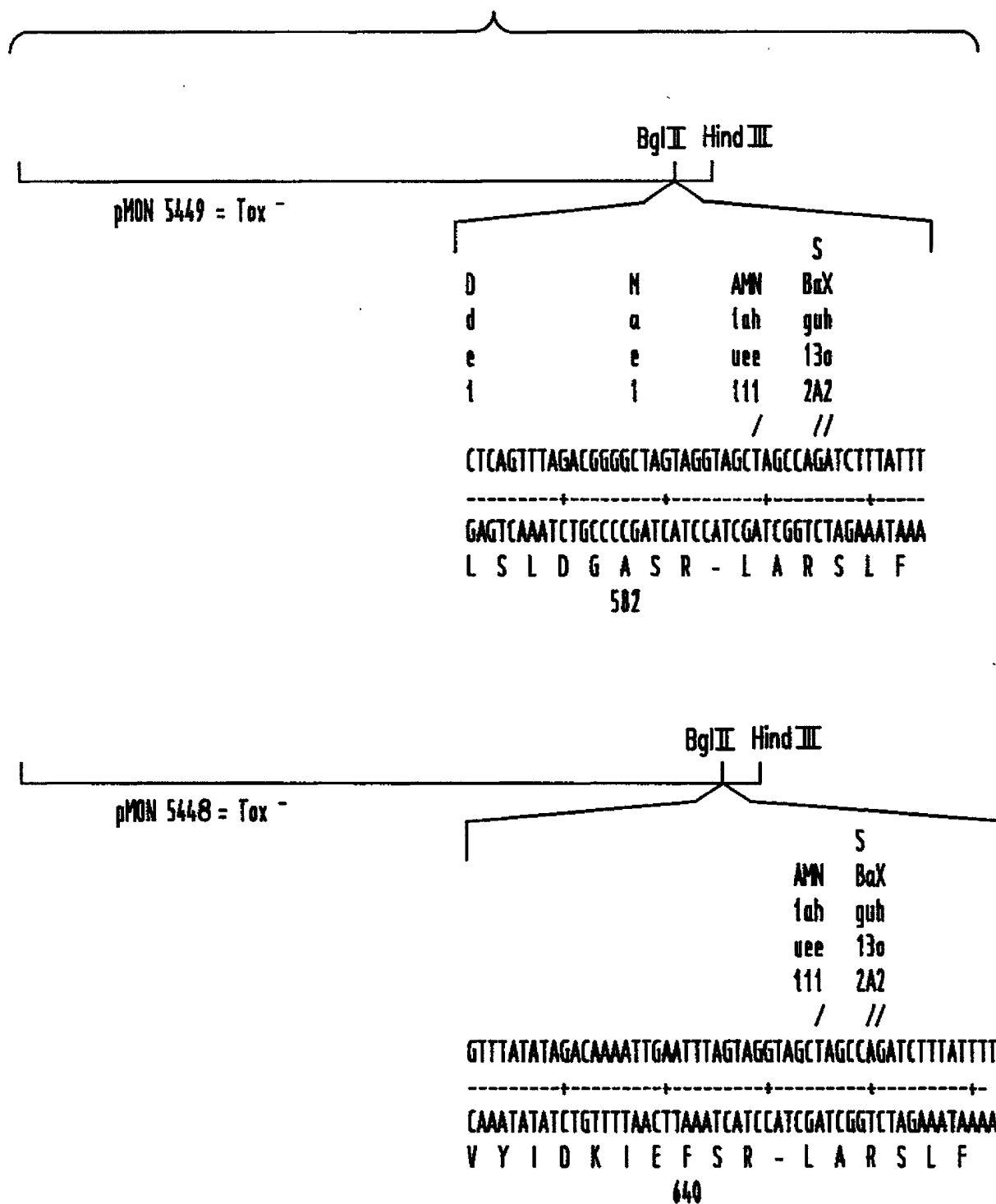
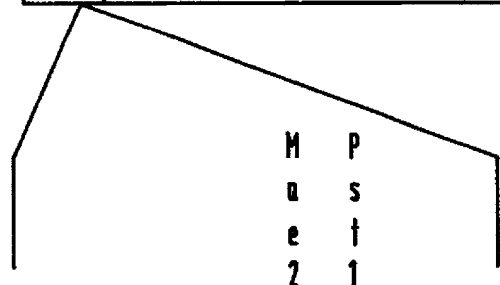
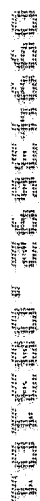


FIG.8A



THE INSERTS SHOW THE ACTUAL AMINO ACID
SEQUENCE OF THE ALTERED B.t.t. PROTEINS.

FIG.8B

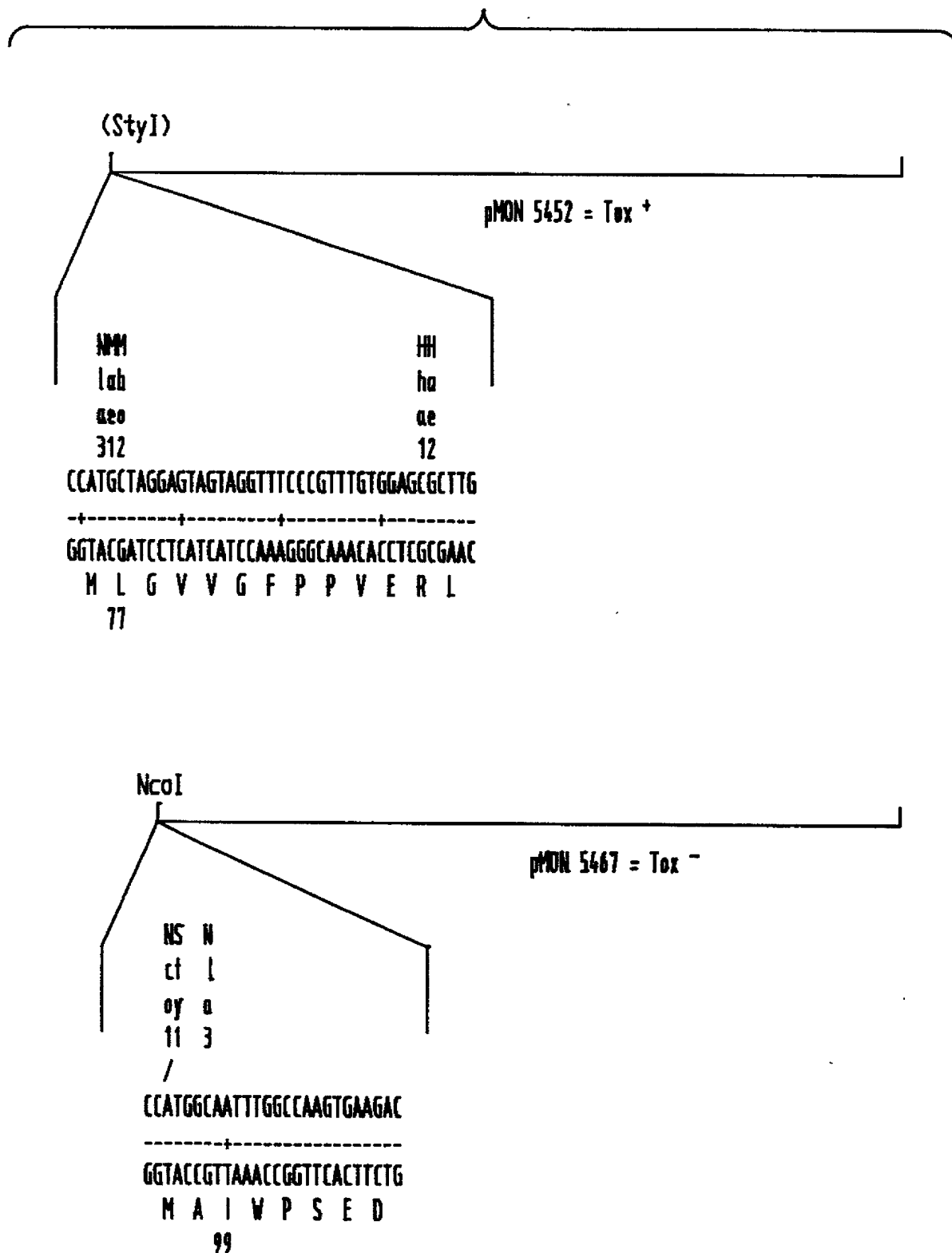


40



48

$$\underbrace{\hspace{10cm}}$$



THE INSERTS SHOW THE ACTUAL AMINO ACID
SEQUENCE OF THE ALTERED B.t.f. PROTEINS.

FIG.9B

[illegible]

FIG. 10

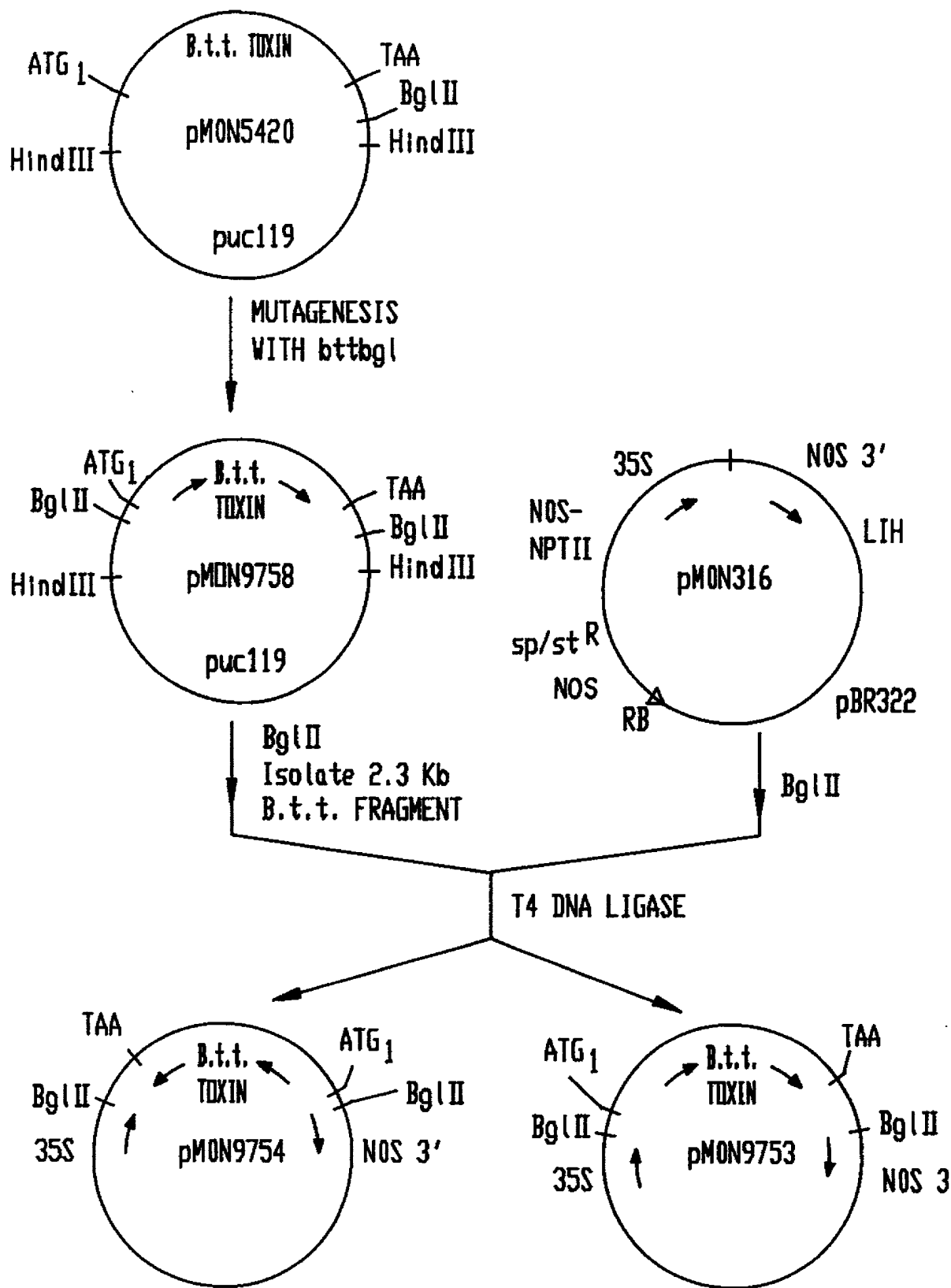


FIG.11

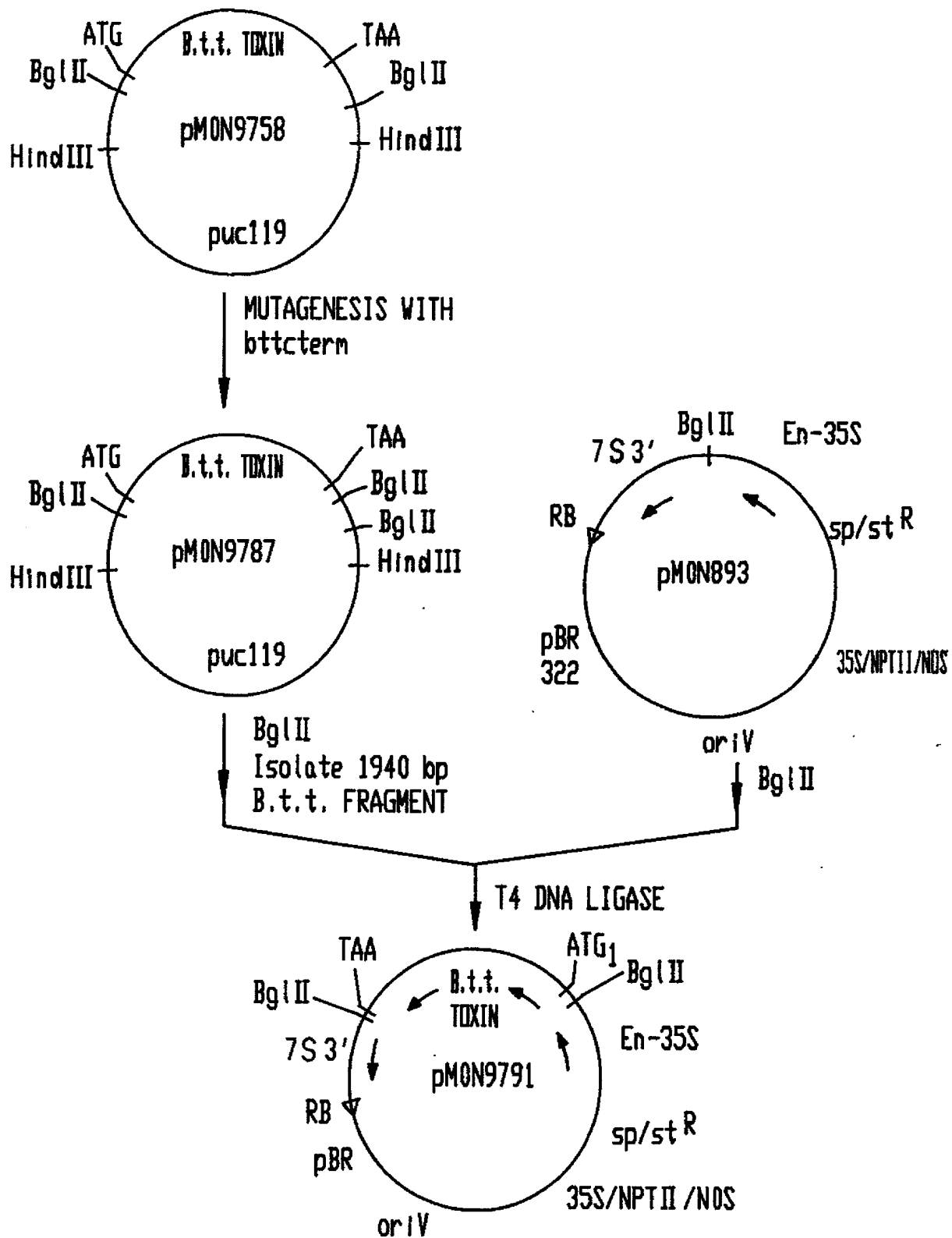


FIG.12

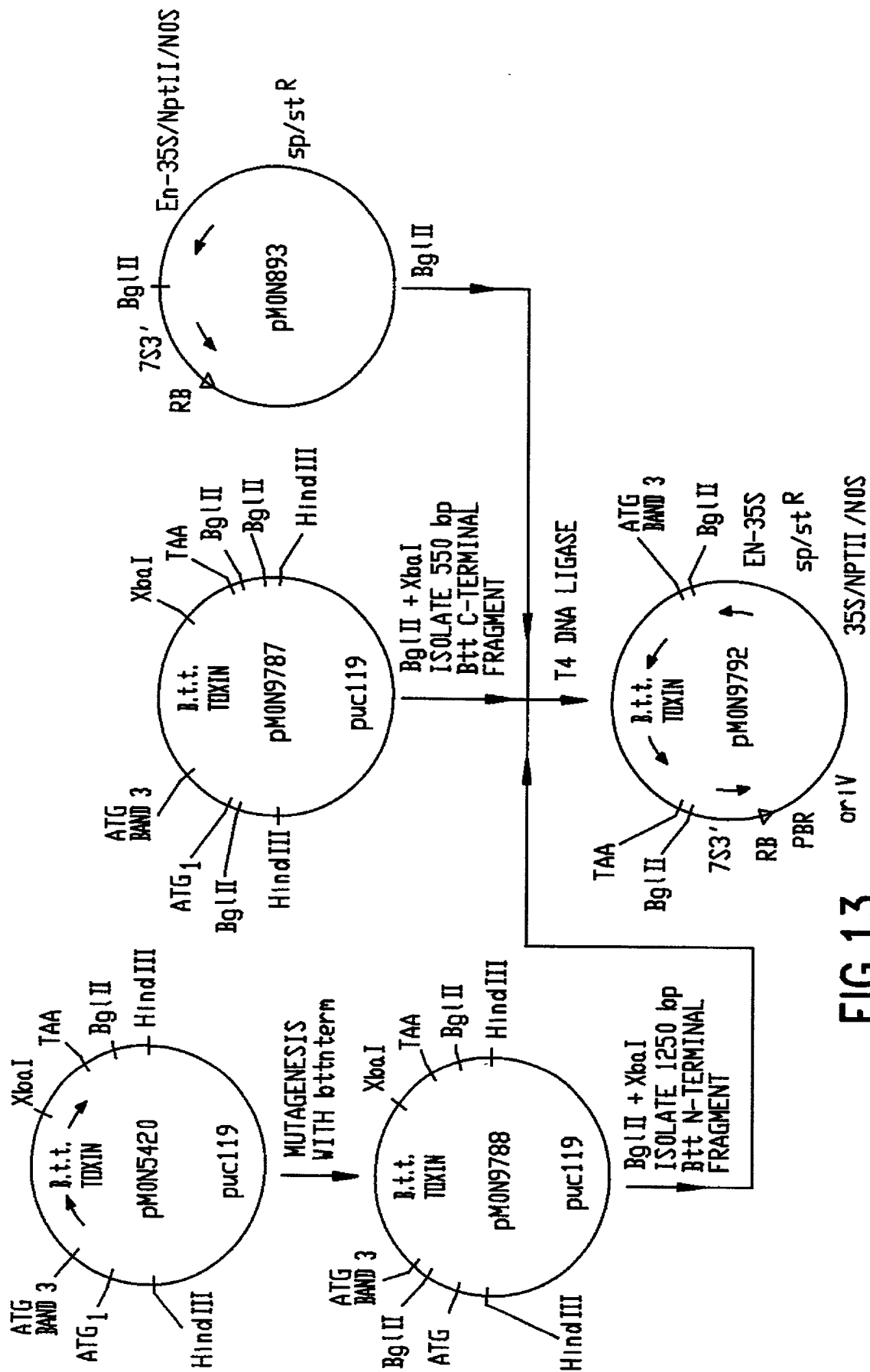


FIG.13

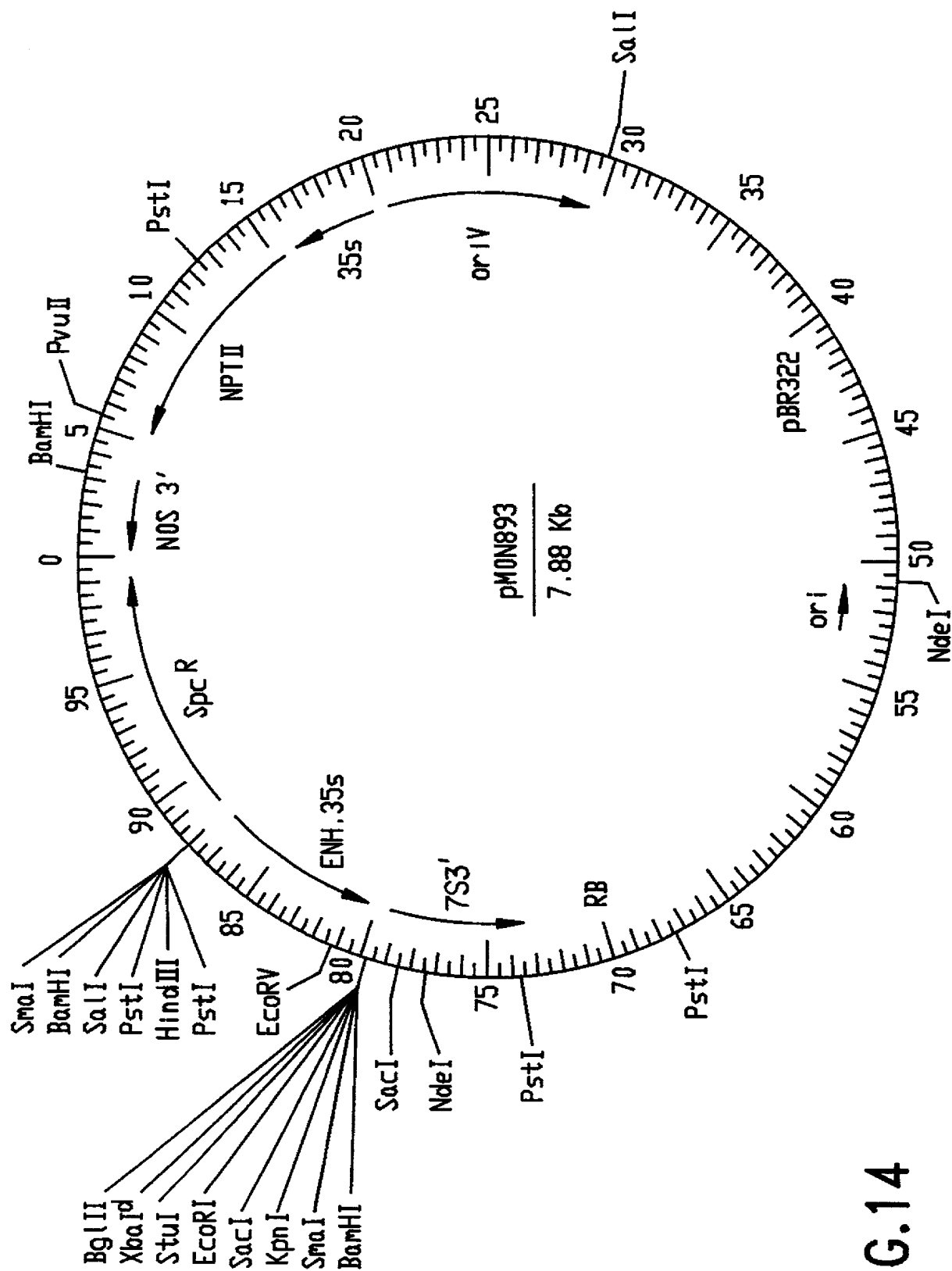


FIG. 14

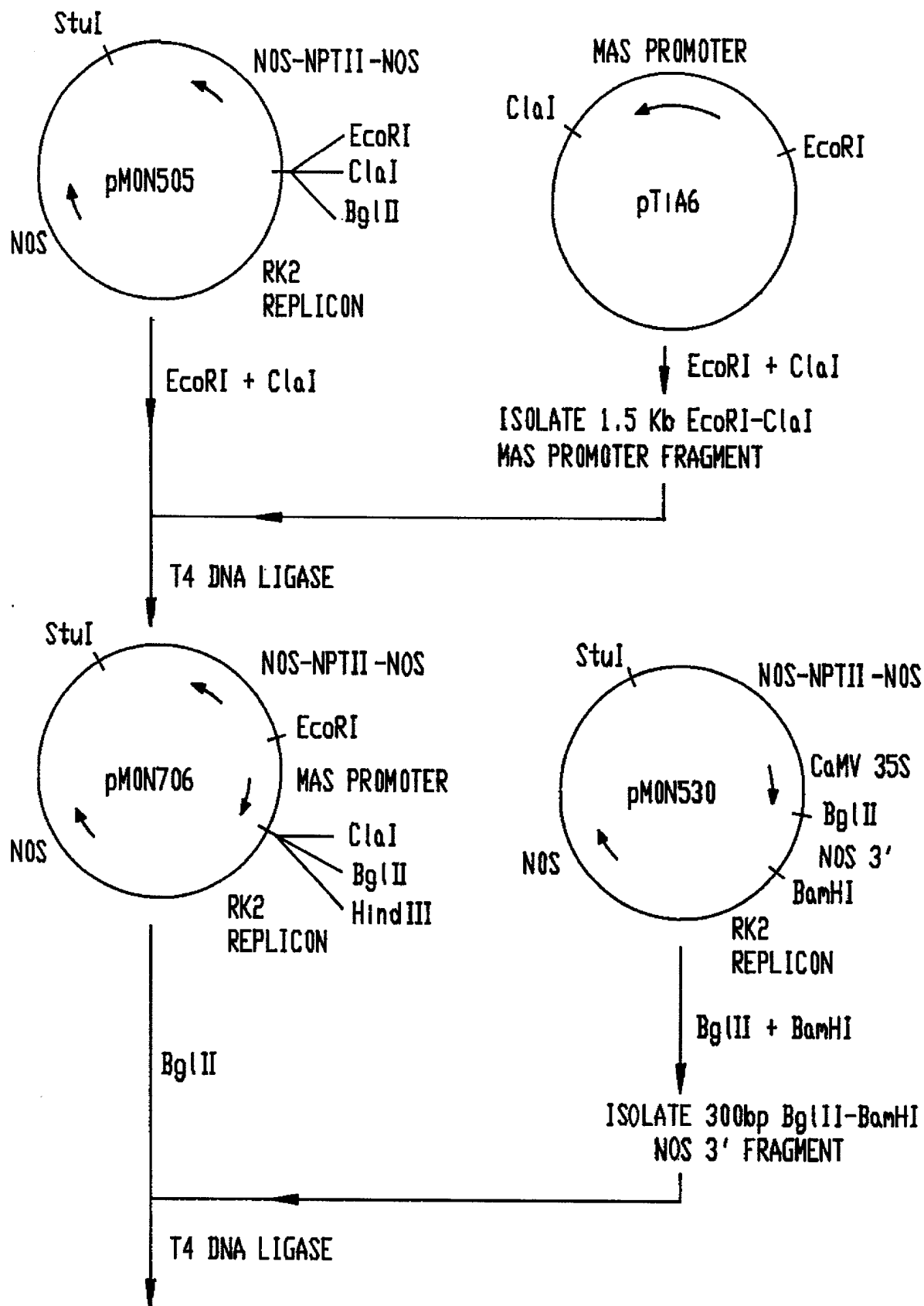


FIG.15A

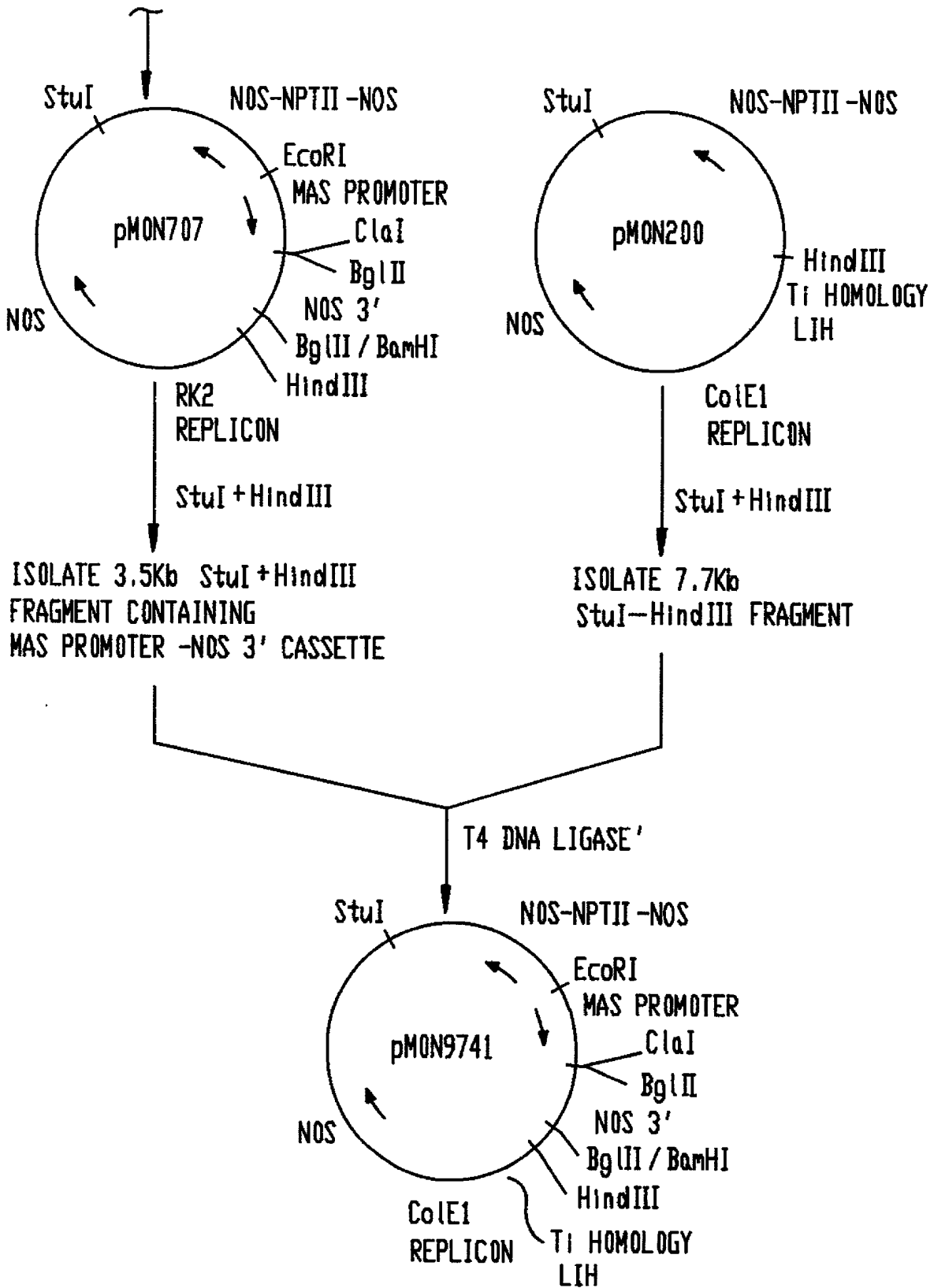


FIG.15B

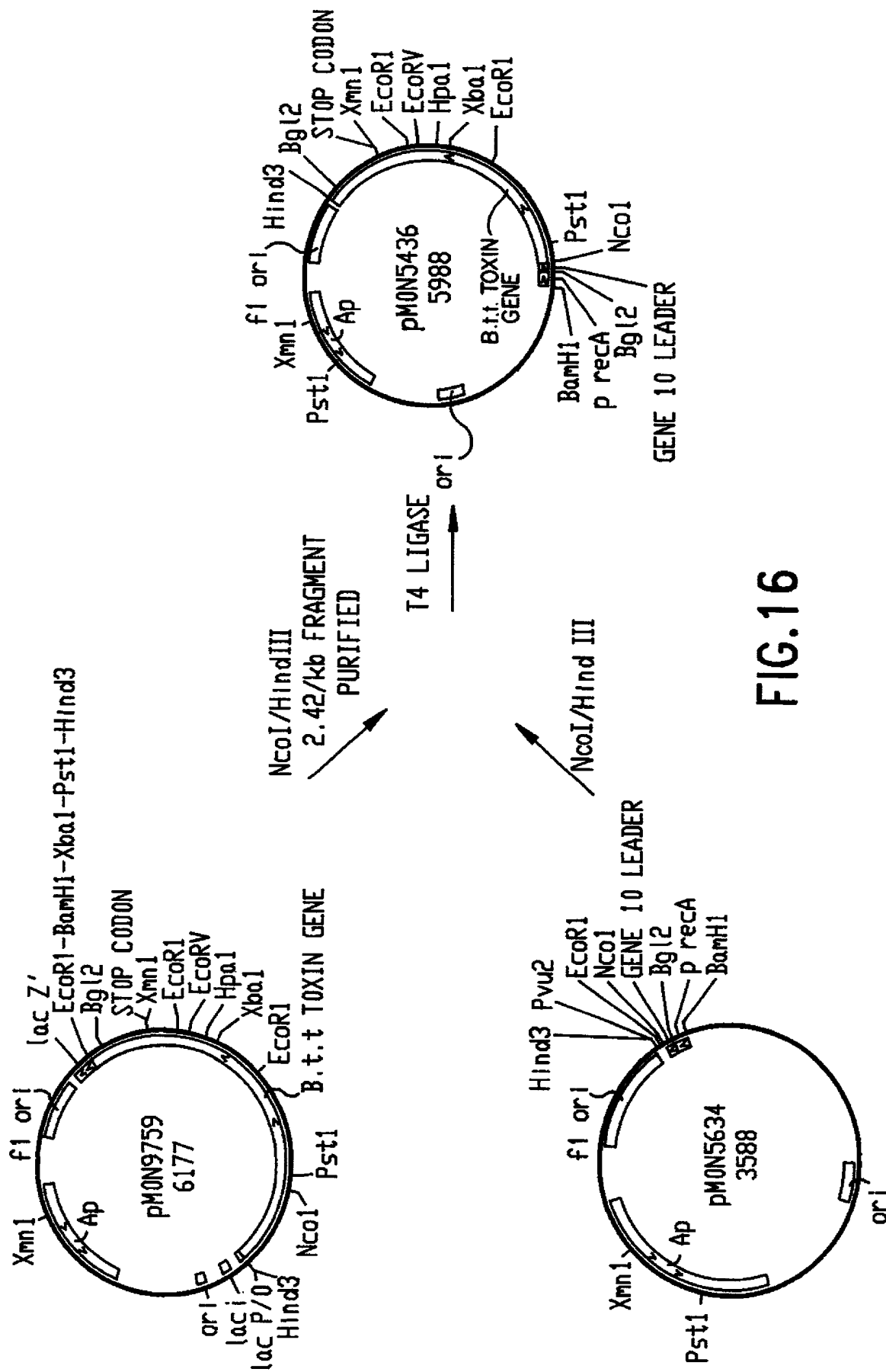


FIG.16

T- DNA REGION IN AGROBACTERIUM ACO

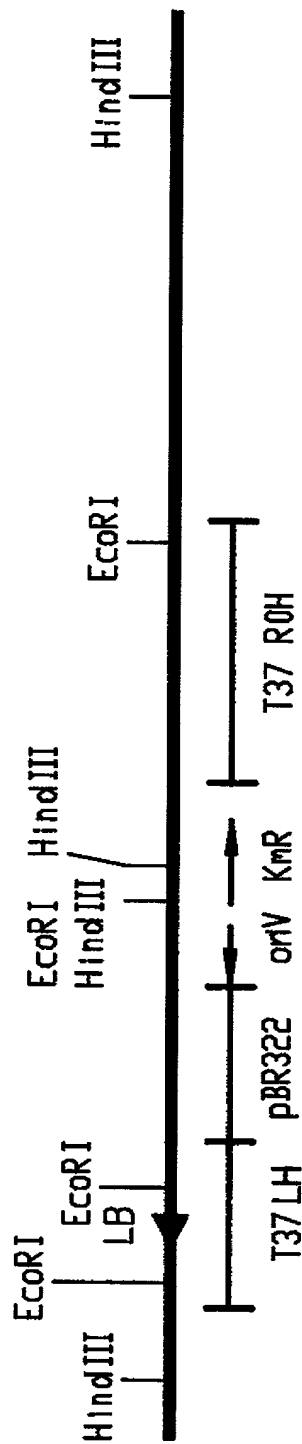


FIG.17

DNA SEQUENCE FOR THE ENHANCED CMV35S PROMOTER
USED IN THE PREPARATION OF pMON893

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5' -AAGCTTGCAT GCCTGCAGGT CCGATG[GAG ACTTTTCAAC AAAGGGTAAT  50
    ATCCGGAAAC CTCCTCGGAT TCCATTGCCC AGCTATCTGT CACTTTATTG  100
    TGAAGATAGT GGAAAAGGAA GGTGGCTCCT ACAAATGCCA TCATTGGCAT  150
    AAAGGAAAGG CCATCGTTGA AGATGECTCT GCGACAGTG GTCCCAAAGA  200
    TGGACCCCCA CCCACGAGGA GCATCGTGA AAAAGAAGAC GTTCCAACCA  250
    CGTCTTCAA GCAAGTGGAT TGATGTGATG GTCCGATG[AGACTTTTCA  300
    ACAAAGGTA ATATCCGGAA ACCTCCTCGG ATTCATTGC CCAGCTATCT  350
    GTCACTTTAT TGTGAAGATA GTGGAAAAGG AAGGTGGCTC CTACAAATGC  400
    CATCATTGCG ATAAAGGAAA GGCCATCGTT GAAGATGCCT CTGCCGACAG  450
    TGGTCCCAA GATGGACCC CACCCACGAG GAGCATCGTG GAAAAAGAAG  500
    ACGTTCCAAC CACGTCTTCA AAGCAAGTGG ATTGATGTGA ]ATCTCCACT  550
    GACGTAAGGG ATGACGCACA ATCCCACTAT CCTTCGCAAG ACCCTTCCTC  600
    TATATAAGGA AGTTCATTTC ATTTGGAGAG GACACGCTGA CAAGCTGACT  650
    CTAGCAGATC T - 3'                                           661
  
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* BRACKETED SEQUENCE INDICATED DUPLICATED ENHANCER SEQUENCE

FIG.18